PROTEIN VISUALIZATION

“BLUE SILVER” COOMASSIE COLLOIDAL BLUE STAIN

Before staining, **Fixation with:**
- 30% Methanol
- 10% Acetic Acid

Incubate the gels in fixation solution for 30 minutes to 1 hour.
After fixation wash the gels 4 times with distilled water (15 minutes for each wash).

The final concentrations adopted in the working colloidal “blue silver” solution are:
- 0.12% dye
- 10% ammonium sulfate
- 10% phosphoric acid
- 20% methanol

This produces a dark green dye solution, which turns to a deep blue when adsorbed onto the polypeptide chains fixed in the polyacrylamide gel, or blotted onto membranes. The dye solution is prepared as follows, by sequentially adding the various ingredients as here indicated:

To a water solution (1/10 of the final volume) the desired amount of phosphoric acid is added, so that, in the final volume, its concentration will be 10%; to this, add the required amount of ammonium sulfate (in powder), calculated to obtain a final concentration of 10%. When the ammonium sulfate has dissolved, add enough Coomassie Blue G-250 (in powder) to obtain a final concentration of 0.12%. When all solids have dissolved, add water to 80% of the final volume. To this solution, under stirring, add anhydrous methanol to reach a 20% final concentration. This stock dye solution should be kept in a brown bottle and is stable at room temperature for >6 months.

*** For staining use 5 times the volume of the gel. For instance for a maxi gel (~50 mL of SDS-PAGE solution) you have to use about 200-250mL of this blue silver coomassie colloidal stain.
Keep the gels in staining solution for at least 3 hours, better overnight.

Destain with distilled water, change water 3-4 times.